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The application was originally filed in English.

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NEW FORMULATION

FIELD OF THE INVENTION

The present invention relates to pharmaceutical formulations of an inhibitor of carboxypeptidase U (CPU) and a thrombin inhibitor in admixture with a pharmaceutically acceptable adjuvant, diluent and/or carrier, as well as a kit of parts, a method for treatment and use of the formulations for the treatment or prophylaxis of a condition in which inhibition of CPU and/or inhibition of thrombin are required or desired.

BACKGROUND OF THE INVENTION

Blood coagulation is the key process involved in both haemostasis (i.e. prevention of blood loss from a damaged vessel) and thrombosis (i.e. the pathological occlusion of a blood vessel by a blood clot). Coagulation is the result of a complex series of enzymatic reactions, where one of the final steps is conversion of the proenzyme prothrombin to the active enzyme thrombin.

Thrombin plays a central role in coagulation. It activates platelets, it converts fibrinogen into fibrin monomers, which polymerise spontaneously into filaments, and it activates factor XIII, which in turn crosslinks the polymer to insoluble fibrin. Thrombin further activates factor V and factor VIII in a positive feedback reaction. Inhibitors of thrombin are therefore expected to be effective anticoagulants by inhibition of platelets, fibrin formation and fibrin stabilization. By inhibiting the positive feedback mechanism they are expected to excert inhibition early in the chain of events leading to coagulation and thrombosis.

Fibrinolysis is the result of a series of enzymatic reactions resulting in the degradation of fibrin by plasmin. The activation of plasminogen is the central process in fibrinolysis. The cleavage of plasminogen to produce plasmin is accomplished by the plasminogen activators, tissue-type plasminogen activator (t-PA) or urokinase-type plasminogen activator (u-PA). Initial plasmin degradation of fibrin generates carboxy-terminal lysine residues that

serve as high affinity binding sites for plasminogen. Since plasminogen bound to fibrin is much more readily activated to plasmin than free plasminogen this mechanism provides a positive feedback regulation of fibrinolysis.

One of the endogenous inhibitors to fibrinolysis is CPU. CPU is also known as plasma carboxypeptidase B, active thrombin activatable fibrinolysis inhibitor (TAFIa), carboxypeptidase R and inducible carboxypeptidase activity. CPU is formed from its precursor procarboxypeptidase U (proCPU) by the action of proteolytic enzymes *e.g.* thrombin, thrombin-thrombomodulin complex or plasmin. CPU cleaves basic amino acids at the carboxy-terminal of fibrin fragments. The loss of carboxy-terminal lysines and thereby of lysine binding sites for plasminogen then serves to inhibit fibrinolysis.

SUMMARY OF THE INVENTION

The present invention relates to pharmaceutical formulations containing an inhibitor of CPU and a thrombin inhibitor in admixture with a pharmaceutically acceptable adjuvant, diluent and/or carrier, as well as a method for treatment and use of the formulations for the treatment or prophylaxis of a condition in which inhibition of CPU and/or inhibition of thrombin are required or desired.

The invention further relates to a kit of parts of vessels containing the CPU inhibitor and the thrombin inhibitor and instructions for the administration of the inhibitors to a patient in need thereof.

The invention also relates to a kit of parts of formulations containing the CPU inhibitor and the thrombin inhibitor each in admixture with a pharmaceutically acceptable adjuvant, diluent and/or carrier.

DETAILED DESCRIPTION OF THE INVENTION

It has surprisingly been found that, compared to the use of CPU and thrombin inhibitors separately, the present invention comprising a combination of a CPU inhibitor and a thrombin inhibitor potentiate anti-thrombotic effects, thereby reducing the risk for thrombosis and hypercoagulability in blood and tissues of mammals. Furthermore, the present invention offers administration of lower doses of the active ingredients, thereby reducing the risk of side effects, e.g. bleeding complications.

In one aspect, the present invention thus relates to pharmaceutical formulations, comprising:
(i) an inhibitor of carboxypeptidase U or a pharmaceutically acceptable salt thereof, and
(ii) a thrombin inhibitor or a pharmaceutically acceptable salt thereof, in admixture with a pharmaceutically acceptable adjuvant, diluent and/or carrier.

A further aspect of the present invention relates to kits of parts comprising:
(i) a vessel containing an inhibitor of carboxypeptidase U, or a pharmaceutically acceptable salt thereof;
(ii) a vessel containing a thrombin inhibitor, or a pharmaceutically acceptable salt thereof; and instructions for the sequential, separate and/or simultaneous administration of the inhibitors (i) and (ii) to a patient in need thereof.

Another aspect of the invention relates to kits of parts comprising:
(i) a pharmaceutical formulation containing an inhibitor of carboxypeptidase U, or a pharmaceutically acceptable salt thereof, in admixture with a pharmaceutically acceptable adjuvant, diluent and/or carrier; and
(ii) a pharmaceutical formulation containing a thrombin inhibitor, or a pharmaceutically acceptable salt thereof, in admixture with a pharmaceutically acceptable adjuvant, diluent and/or carrier;
which inhibitors (i) and (ii) are each provided in a form that is suitable for administration in conjunction with the other.

By "administration in conjunction with", we include that respective formulations comprising a CPU inhibitor and a thrombin inhibitor are administered, sequentially, separately and/or simultaneously, over the course of treatment of the relevant condition, which condition may be acute or chronic. Preferably, the term includes that the two formulations are administered (optionally repeatedly) sufficiently closely in time for there to be a beneficial effect for the patient, that is greater, over the course of the treatment of the relevant condition, than if either of the two formulations are administered (optionally repeatedly) alone, in the absence of the other formulation, over the same course of treatment. Determination of whether a combination provides a greater beneficial effect in respect of, and over the course of treatment of, a particular condition, will depend upon the condition to be treated or prevented, but may be achieved routinely by the poerson skilled in the art.

Thus, the term "in conjunction with" includes that one or other of the two formulations may be administered (optionally repeatedly) prior to, after, and/or at the same time as, administration with the other component. When used in this context, the terms "administered simultaneously" and "administered at the same time as" include that individual doses of CPU inhibitor and thrombin inhibitor are administered within 48 hours, e.g. 24 hours, of each other.

Yet another aspect of the invention relates to methods for treatment or prophylaxis of a patient suffering from, or susceptible to, a condition in which inhibition of thrombin and/or inhibition of carboxypeptidase U are required or desired, which method comprises administering to the patient a therapeutically effective total amount of

(i) an inhibitor of carboxypeptidase U, or a pharmaceutically acceptable salt thereof, in admixture with a pharmaceutically acceptable adjuvant, diluent and/or carrier; in conjunction with

(ii) a thrombin inhibitor, or a pharmaceutically acceptable salt thereof, in admixture with a pharmaceutically acceptable adjuvant, diluent and/or carrier.

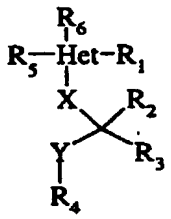
Still another aspect of the invention relates to methods for treatment or prophylaxis of a patient suffering from, or susceptible to, a condition in which inhibition of thrombin and/or inhibition of carboxypeptidase U are required or desired, which method comprises administering to the patient a pharmaceutical formulation, comprising:
(i) an inhibitor of carboxypeptidase U or a pharmaceutically acceptable salt thereof, and
(ii) a thrombin inhibitor or a pharmaceutically acceptable salt thereof, in admixture with a pharmaceutically acceptable adjuvant, diluent and/or carrier.

A still further aspect of the invention relates to the use of pharmaceutical formulations, comprising:
(i) an inhibitor of carboxypeptidase U or a pharmaceutically acceptable salt thereof, and
(ii) a thrombin inhibitor or a pharmaceutically acceptable salt thereof, in admixture with a pharmaceutically acceptable adjuvant, diluent and/or carrier,
in the manufacture of a medicament for the treatment or prophylaxis of a condition in which inhibition of thrombin and/or inhibition of carboxypeptidase U are required or desired.

CPU inhibitors

Inhibitors of CPU referred to in this application include low molecular weight inhibitors of carboxypeptidase with a molecular weight below about 1000, suitably below 700.

In the present application, the CPU inhibitor is preferably
(i) a compound of general formula I



I

or a pharmaceutically acceptable salt thereof, wherein

R_1 represents H, OH, halogen, C_1-C_6 alkyl, C_1-C_6 alkylaryl, C_1-C_6 alkoxy, cyano, NO_2, SH, $N(R_8)_2$, or CO-C_1-C_6 alkyl,

R_2 represents H, OH, halogen, or C_1-C_6 alkyl,

R_3 represents $COOR_7$, SO_2R_7, $S{=}O(OR_7)$, $P{=}O(OR_7)_2$, $B(OR_7)_2$, $P{=}OR_7(OR_7)$, tetrazole or any carboxylic acid isostere,

R_4 represents SH, S-CO- C_1-C_6 alkyl, S-CO-alkylaryl or S-CO-aryl,

R_5 represents H, OH, halogen, C_1-C_6 alkyl, C_1-C_6 alkylaryl, C_1-C_6 alkoxy, cyano, NO_2, SH, $N(R_8)_2$, or CO-C_1-C_6 alkyl,

R_6 represents H, OH, halogen, C_1-C_6 alkyl, C_1-C_6 alkylaryl, C_1-C_6 alkoxy, cyano, NO_2, SH, $N(R_8)_2$, or CO-C_1-C_6 alkyl,

R_7 represents H, C_1-C_6 alkyl, C_1-C_6 alkylaryl, or aryl,

R_8 represents H or C_1-C_6 alkyl,

X represents O, S, $C(Z)_2$, N(Z), NR_8CO or $CONR_8$,

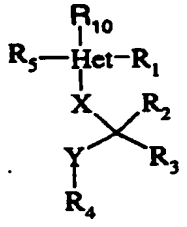
Y represents CH_2, or CH(Z),

Z represents H, C_1-C_6 alkyl, aryl or C_1-C_6 alkylaryl, and

Het represents a 4-, 5-, or 6-membered aromatic or alifatic heterocyclic group containing at least one nitrogen, oxygen or sulphur atom or a 4-, 5-, or 6-membered aromatic or alifatic carbocyclic group,

or

(ii) a compound of general formula II



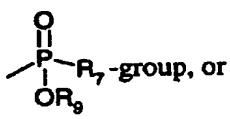
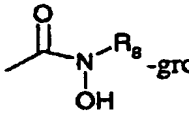
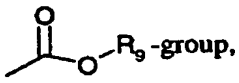
II

or a pharmaceutically acceptable salt thereof, wherein

R_1 represents H, OH, halogen, C_1-C_6 alkyl, C_1-C_6 alkylaryl, C_1-C_6 alkoxy, cyano, NO_2, SH, $N(R_8)_2$, CO-C_1-C_6 alkyl, or a guanidino group,

R_2 represents H, OH, halogen, or C_1-C_6 alkyl,

R_3 represents $COOR_6$, SO_2R_6, SO_3R_6, $P{=}O(OR_6)_2$, $B(OR_6)_2$, $P{=}OR_6(OR_6)$, tetrazole or any carboxylic acid isostere,

R_4 represents a  -group, or a  -group, or a  -group,

R_5 represents H, OH, halogen, C_1-C_6 alkyl, C_1-C_6 alkylaryl, C_1-C_6 alkoxy, cyano, NO_2, SH, $N(R_8)_2$, CO-C_1-C_6 alkyl, or a guanidino group,

R_6 represents H, C_1-C_6 alkyl, C_1-C_6 alkylaryl, or aryl,

R_7 represents C_1-C_6 alkyl, C_1-C_6 alkylaryl, aryl or a dipeptide or an aminoacid residue, both optionally N-protected,

R_8 represents H, C_1-C_6 alkyl, or C_1-C_6 alkylaryl,

R_9 represents H, C_1-C_6 alkyl, C_1-C_6 alkylaryl, aryl or $OC(Z)_2OCOR_8$,

R_{10} represents H, OH, halogen, C_1-C_6 alkyl, C_1-C_6 alkylaryl, C_1-C_6 alkoxy, cyano, NO_2, SH, $N(R_8)_2$, CO-C_1-C_6 alkyl, or a guanidino group,

X represents O, S, CH_2, CH_2CH_2, $CH_2CH_2CH_2$, NH, CH(Z) or N(Z),

Y represents O, CH_2, or CH(Z), or a single bond,

Z represents H, C_1-C_6 alkyl, C_1-C_6 alkylaryl or aryl, and

Het represents a 4-, 5-, or 6-membered aromatic or alifatic heterocyclic group containing at least one nitrogen, oxygen or sulphur atom, a 4-, 5-, or 6-membered aromatic or alifatic carbocyclic group or a single bond.

More preferred inhibitors of CPU includes compounds of general formula I, or a pharmaceutically acceptable salt thereof, wherein the following individual and separate preferences apply

R_1 represents H, OH, NH_2, halogen, C_1-C_6 alkyl, or C_1-C_6 alkoxy,

R_2 represents H, OH, halogen, or C_1-C_6 alkyl,

R_3 represents $COOR_7$,

R_4 represents SH, S-CO-C_1-C_6 alkyl, S-CO-alkylaryl or S-CO-aryl,

R_5 represents H, OH, NH_2, halogen, C_1-C_6 alkyl, or C_1-C_6 alkoxy,

R_6 represents H, OH, NH_2, halogen, C_1-C_6 alkyl, or C_1-C_6 alkoxy,

R_7 represents H, C_1-C_6 alkyl, C_1-C_6 alkylaryl or aryl,

X represents O, S, CH_2 or NH,

Y represents CH_2,

Het represents a 4-, 5- or 6-membered aromatic or alifatic heterocyclic group containing at least one nitrogen atom or a 4-, 5- or 6-membered aromatic or alifatic carbocyclic group.

Even more preferred inhibitors of CPU includes compounds of general formula I, or a pharmaceutically acceptable salt thereof, wherein the following individual and separate preferences apply

R_1 represents H, NH_2, halogen, or C_1-C_6 alkyl,

R_2 represents H, or C_1-C_6 alkyl,

R_3 represents $COOR_7$,

R_4 represents SH, or S-CO-C_1-C_6 alkyl,

R_5 represents H, NH_2, halogen, or C_1-C_6 alkyl,

R_6 represents H, NH_2, halogen, or C_1-C_6 alkyl,

R_7 represents H, or C_1-C_6 alkyl,

X represents CH_2,

Y represents CH_2,

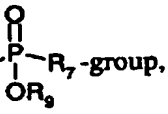
Het represents pyridyl or piperidinyl.

More preferred inhibitors of CPU includes compounds of general formula II, or a pharmaceutically acceptable salt thereof, wherein the following individual and separate preferences apply

R_1 represents H, NH_2, or a guanidino group,

R_2 represents H,

R_3 represents $COOR_6$,

R_4 represents a  -group,

R_5 represents H, NH_2, or a guanidino group,

R_6 represents H, or C_1-C_6 alkyl,

R_7 represents C_1-C_6 alkyl or C_1-C_6 alkylaryl, or a dipeptide or an aminoacid residue, both optionally N-protected,

R_8 represents H, C_1-C_6 alkyl, or C_1-C_6 alkylaryl,

R_9 represents H, C_1-C_6 alkyl, C_1-C_6 alkylaryl, aryl or $OC(Z)_2OCOR_8$,

R_{10} represents H, NH_2, or a guanidino group,

X represents CH_2, CH_2CH_2, or $CH_2CH_2CH_2$,

Y represents O, CH_2 or CH(Z),

Z represents H, C_1-C_6 alkyl, C_1-C_6 alkylaryl or aryl,

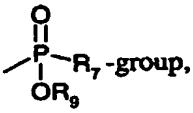
Het represents pyridyl, piperidinyl or a single bond.

Even more preferred inhibitors of CPU includes compounds of general formula II, or a pharmaceutically acceptable salt thereof, wherein the following individual and separate preferences apply

R_1 represents H, or NH_2,

R_2 represents H,

R_3 represents $COOR_6$,

R_4 represents a  -group,

R_5 represents H, or NH_2,

R_6 represents H, or C_1-C_6 alkyl,

R_7 represents a dipeptide or an aminoacid residue, both optionally N-protected,

R_8 represents H, C_1-C_6 alkyl, or C_1-C_6 alkylaryl,

R_9 represents H, C_1-C_6 alkyl, C_1-C_6 alkylaryl, aryl or $OC(Z)_2OCOR_8$,

R_{10} represents H, or NH_2,

X represents CH_2, or CH_2CH_2,

Y represents CH_2,

Z represents H, or C_1-C_6 alkyl,

Het represents pyridyl or piperidinyl.

The following definitions shall apply throughout the specification and the appended claims:

The term "C_1–C_6 alkyl" denotes a straight or branched, substituted or unsubstituted alkyl group having from 1 to 6 carbon atoms. Examples of said alkyl include, but is not limited to methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, sec-butyl, t-butyl and straight- and branched-chain pentyl and hexyl.

The term "C_1-C_6 alkoxy" denotes a group O-alkyl, wherein alkyl is as defined above.

The term "4-, 5-, or 6-membered aromatic or alifatic heterocyclic group containing at least one nitrogen, oxygen or sulphur atom" includes, but is not limited to substituted or unsubstituted azetidine, furan, thiophene, pyrrole, pyrroline, pyrrolidine, dioxolane, oxathiolane, oxazolane, oxazole, thiazole, imidazole, imidazoline, imidazolidine, pyrazole, pyrazoline, pyrazolidine, isoxazole, isothiazole, oxadiazole, furazan, triazole, thiadiazole, pyran, pyridine, piperidine, dioxane, morpholine, dithiane, oxathiane, thiomorpholine, pyridazine, pyrimidine, pyrazine, piperazine, triazine, thiadiazine, dithiazine groups, and shall be understood to include all isomers of the above identified groups. The term "azetidinyl" shall for example by understood to include the 2-, and 3-isomers and the terms "pyridyl" and "piperidinyl" shall for example by understood to include the 2-, 3-, and 4-isomers.

The term "4-, 5-, or 6-membered aromatic or alifatic carbocyclic group" includes, but is not limited to substituted or unsubstituted phenyl, cyclobutyl, cyclopentyl, cyclohexyl, cyclobutenyl, cyclopentenyl, cyclohexenyl, cyclopentadienyl, cyclohexadienyl groups

The term "halogen" includes fluoro, chloro, bromo and iodo groups.

The term "aryl" denotes a substituted or unsubstituted C_6–C_{14} aromatic hydrocarbon and includes, but is not limited to, benzene, naphthalene, indene, anthracene, fenantrene, and fluorene.

The term "dipeptide or aminoacid residue" denotes a dipeptid or an aminoacid excluding the C-terminal carboxyl group.

The term "substituted" denotes an C_1-C_6 alkyl, C_1-C_6 alkylaryl or aryl group as defined above which is substituted by one or more alkyl, alkoxy, halogen, amino, thiol, nitro, hydroxy, acyl or cyano groups.

The pure enantiomers, racemic mixtures and unequal mixtures of two enantiomers are all within the scope of the present invention. It should also be understood that all of the diastereomeric forms possible are within the scope of the invention.

It should also be understood that all polymorphs, amorphous forms, anhydrates, hydrates, solvates of the compounds are within the scope of the invention.

Depending on the process conditions the compounds of general formula I and II may be obtained either in neutral form or as pharmaceutically acceptable salts, all of which are within the scope of the present invention.

Also included in the invention are derivatives of the compounds of the formula I and II which have the biological function of the compounds of the formula I and II, respectively. For example, certain protected derivatives of compounds of formula I or II may be made prior to a final deprotection stage to form compounds of formula I and II, respectively.

The use of protecting groups is described in 'Protective Groups in Organic Synthesis', 2nd edition, T.W. Greene & P.G.M. Wutz, Wiley-Interscience (1991). The protective group may also be a polymer resin such as Wang resin or a 2-chlorotrityl chloride resin.

It will also be appreciated by those skilled in the art that, although such protected derivatives of compounds of formula I or II may not possess pharmacological activity as such, they may be administered parenterally or orally, together with the thrombin inhibitor, and thereafter metabolised in the body to form compounds of formula I or II which are pharmacologically active. Such derivatives may therefore be described as "prodrugs". All prodrugs of compounds of formula I or II are included within the scope of the invention.

In the present application, CPU inhibitors include chemical modifications, such as esters, prodrugs and metabolites, whether active or inactive, and pharmaceutically acceptable salts of any of these.

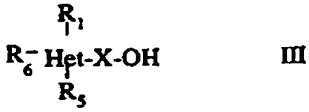
Preparation of the CPU inhibitors of general formula I

The CPU inhibitors of general formula I may be prepared using the following processes A-C.

Process A

Process A for the manufacture of compounds with the general formula I, wherein R_1, R_4, R_5, R_6, R_7, Y and Het are as defined above and R_2 is H, R_3 is $COOR_7$, and X is CH_2, comprises the following steps:

a) Compounds of the general formula III



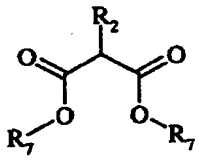
III

wherein R_1, R_5, R_6 and Het are as defined for formula I and X is CH_2,which are either commercially available or are available using known techniques, can be converted into a compound of the general formula IV,

IV

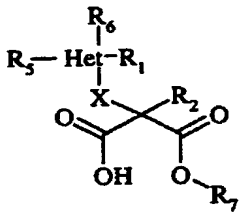
wherein L is a suitable leaving group, such as chloro, bromo, iodo, triflate or tosyl, under standard conditions using a suitable reagent, such as PPh_3/CBr_4, TosCl/pyridine or $(CF_3SO_2)_2O$/TEA).

b) Compounds of the general formula IV can thereafter be reacted with compounds of the general formula V



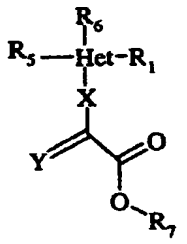
V

wherein R_2 and R_7 are as defined for formula I, which are either commercially available, or are available using known techniques, in the presence of a suitable base, such as K_2CO_3 or NaH, under standard conditions to give compounds of the general formula VI.



VI

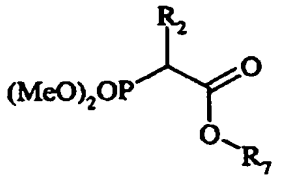
c) Compounds of the general formula VI can thereafter be converted to compounds of the general formula VII



VII

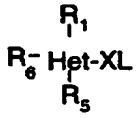
by treatment with formaldehyd in the presence of a suitable base, such as Et_2NH, under standard conditions.

However, if Y is CH(Z) then compounds of the general formula VII can be prepared by treating compounds of the general formula VIII



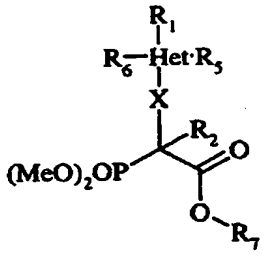
VIII

wherein R_2 and R_7 are as defined for formula I, with an alkylating agent of the general formula IV



IV

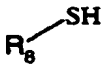
in the presence of a suitable base, such as LDA or NaH, under standard conditions to give compounds of the general formula IX



IX

Compounds of the general formula IX can thereafter be reacted with an appropriate aldehyde CHO(Z), in the presence of a suitable base, such as KOtBu, LDA or NaH, under standard conditions to give a compound of the general formula VII.

d) Compounds of the general formula VII can be further reacted with compounds of the general formula X



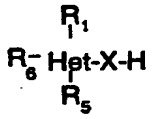
X

wherein R_8 is a suitable protecting group, such as Ac, Bz, PMB or Bn, alone or in the presence of a suitable base, such as NaOMe, K_2CO_3 or triethylamine or alternatively in the presence of a free-radical initiator, such as AIBN under standard conditions to give compounds of the general formula I, wherein R_1, R_4, R_5, R_6, R_7, Y and Het are as defined for formula I and R_2 is H, R_3 is $COOR_7$, and X is CH_2.

Process B

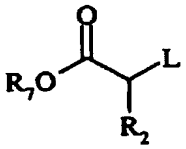
Process B for the manufacture of compounds with the general formula I, wherein R_1, R_2, R_4, R_5, R_6, R_7, and Het, are as defined in formula I and Y is CH_2, R_3 is $COOR_7$, and X is O, S, CH_2, NH or N(Z), comprises the following steps:

a) Reacting a compound of the general formula XI,



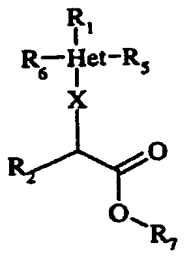
XI

wherein R_1, R_5, R_6, and Het are as defined for formula I and X is O, S, NH or N(Z), with an alkylating agent of the general formula XII,



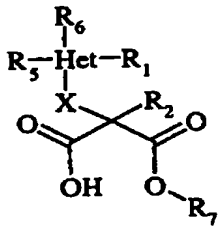
XII

wherein R_2 and R_7 are as defined for formula I and L is a suitable leaving group, such as a chloro, bromo, iodo, triflate or tosylate group, under standard conditions using suitable reagents, such as NaH, Ag_2CO_3, or Bu_4NHSO_4/NaOH, to give compounds of the general formula XIII,



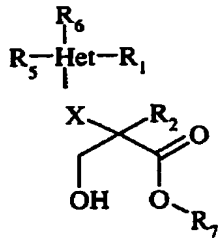
XIII

b) Compounds of the general formula XIII can thereafter be reacted with carbon dioxide in the presence of a suitable base, such as LDA or KHMDS under standard conditions to give a compound of the general formula XIV,



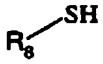
XIV

(c) Compounds of the general formula XIV can thereafter be reacted with ClCOOMe in the presence of a base, such as triethylamine, and thereafter reducing the formed mixed anhydride with a suitable reducing agent, such as $NaBH_4$, under standard conditions, to give a compound of the general formula XV



XV

(d) Compounds of the general formula XV may thereafter be reacted with a compound of the general formula X



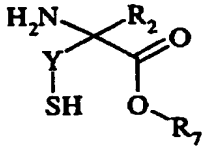
X

wherein R_8 is a suitable protecting group, such as Ac or Bz, in the presence of a suitable reagent, such as PPh_3/DIAD, under standard conditions to give compounds of the general formula I, wherein R_1- R_7 and Het are as defined above and Y is CH_2 and X is O, S, CH_2, NH or N(Z).

Process C

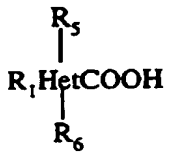
Process C for the manufacture of compounds with the general formula I, wherein R_1, R_2, R_4, R_5, R_6, R_7, Y and Het, are as defined above and R_3 is $COOR_7$ and X is NHCO, comprises the following steps:

a) Reacting a compound of the general formula XVI,



XVI

wherein R_2, R_7, and Y are as defined for formula I with a compound of the general formula XVII,



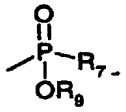
XVII

wherein R_1, R_5, R_6 and Het are as defined for formula I in the presence of suitable coupling reagents, such as PyBOP/DIPEA, DCC/HOBt or EDC/TEA/DMAP under standard conditions to give compounds of the general formula I, wherein R_1, R_2, R_4, R_5, R_6, R_7, and Het, are as defined above and R_3 is $COOR_7$, Y is CH_2 and X is NHCO.

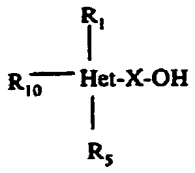
Preparation of the CPU inhibitors of general formula II

The CPU inhibitors of general formula II may be prepared using the following processes D-G.

Process D

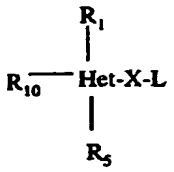
Process D for manufacture of compounds with the general formula II, wherein R_1, R_5, R_6, R_7, R_{10}, Z and Het are as defined above and R_2 is H, R_3 is $COOR_6$, R_4 is a  group, X is CH_2, CH_2CH_2, $CH_2CH_2CH_2$, Y is CH_2 or CH(Z), comprises the following steps:

a) Compounds of the general formula XVIII



XVIII

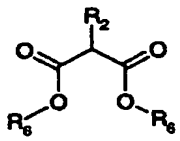
wherein R_1, R_5, R_{10}, X and Het are as defined for general formula II, which are either commercially available or are available using known techniques, can be converted into a compound of the general formula XIX,



XIX

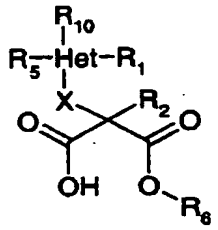
wherein L is a suitable leaving group, such as a chloro, bromo, iodo, triflate or tosyl group, under standard conditions using a suitable reagent, such as PPh_3/CBr_4, TosCl/pyridine or $(CF_3SO_2)_2O$/TEA).

b) Compounds of the general formula XIX can thereafter be reacted with compounds of the general formula XX



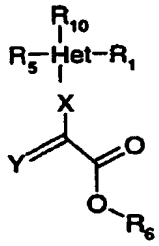
XX

wherein R_2 and R_6 are as defined for general formula II, which are either commercially available or are available using known technics, in the presence of a suitable base, such as K_2CO_3 or NaH, under standard conditions to give compounds of the general formula XXI.



XXI

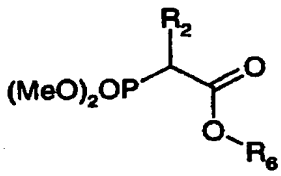
c) Compounds of the general formula XXI can thereafter be converted to compounds of the general formula XXII



XXII

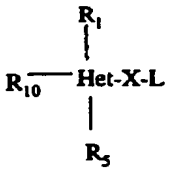
by treatment with formaldehyd in the presence of a suitable base, such as Et_2NH, under standard conditions.

However, if Y is CH(Z) then compounds of the general formula XXII can be prepared by treating compounds of the general formula XXIII



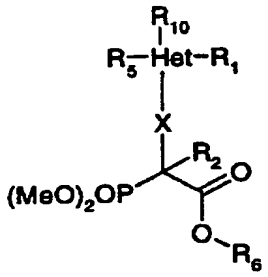
XXIII

wherein R_2 and R_6 are as defined for general formula II, with an alkylating agent of the general formula XIX



XIX

in the presence of a suitable base, such as LDA or NaH, under standard conditions to give compounds of the general formula XXIV

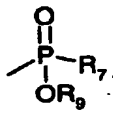


XXIV

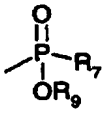
Compounds of the general formula XXIV can thereafter be reacted with an appropriate aldehyde CHO(Z), wherein Z is as defined for general formula II, in the presence of a suitable base, such as KOtBu, LDA or NaH, under standard conditions to give a compound of the general formula XXII.

d) Compounds of the general formula XXII can be further reacted with compounds of the general formula XXV

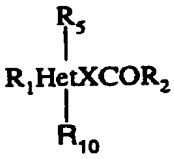
XXV

wherein R_7 is as defined in general formula II, in the presence of a suitable reagent, such as BTSP or HMDS, under standard conditions to give compounds of the general formula II, wherein R_1, R_5, R_6, R_7, R_{10}, Z and Het are as defined above and R_2 is H, R_3 is $COOR_6$, R_4 is a  -group, X is CH_2, CH_2CH_2, $CH_2CH_2CH_2$, Y is CH_2 or CH(Z).

Process E

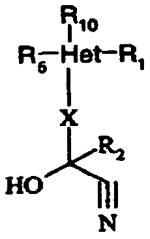
Process E for manufacture of compounds with the general formula II, wherein R_1, R_2, R_3, R_5, R_6, R_7, R_{10}, Z and Het are as defined above, X is CH_2, CH_2CH_2, $CH_2CH_2CH_2$, Y is O, and R_4 is a  -group, comprises the following steps:

a) Reacting a compound of the general formula XXVI



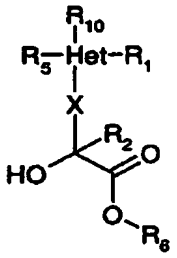
XXVI

wherein R_1, R_2, R_5, R_{10} and Het are as defined in formula II and X is a single bond, CH_2 CH_2CH_2, or $CH_2CH_2CH_2$ in the presence of suitable reagents, such as TMSCN/ZnI_2 or KCN/HOAc, under standard conditions to give compounds of the general formula XXVII



XXVII

b) Compounds of the general formula XXVII can thereafter be treated with suitable reagents, such as HCl or HCl/MeOH, under standard conditions to give compounds of the general formula XXVIII

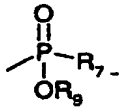


XXVIII

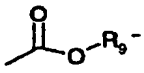
c) Compounds of the general formula XXVIII can thereafter be reacted with compounds of the general formula XXIX,

$$R_7PO_3H_2 \qquad XXIX$$

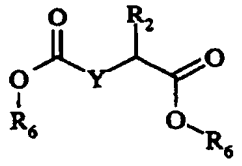
wherein R_7 is as defined in general formula II, which are either commercially available, well known in the literature, or are available using known techniques, in the presence of suitable coupling reagents such a DCC/DMAP, PyBop/DIPEA or $SOCl_2$, under standard conditions to give compounds of the general formula II, wherein R_1, R_2, R_3, R_5, R_6, R_7, R_{10}, Z and Het

are as defined above, X is CH_2, CH_2CH_2, $CH_2CH_2CH_2$, Y is O and R_4 is a  group.

Process F

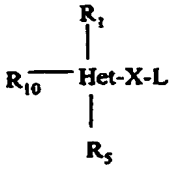
Process F for manufacture of compounds with the general formula II, wherein R_1, R_2, R_5, R_6, R_9,R_{10}, X, Y, Z and Het are as defined above, R_3 is $COOR_6$ and R_4 is a  group, comprises the following steps:

a) reacting a compound of the general formula XXX



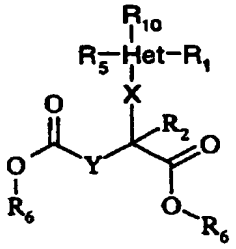
XXX

wherein R_2 and R_6 are as defined in formula II and Y is CH_2, CH(Z), or a single bond, which are either commercially available, well known in the literature, or are available using known techniques, with a compound of the general formula XIX

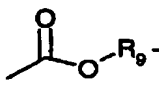


XIX

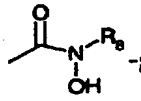
wherein R_1, R_5, R_{10} and Het are as defined for formula II, X is CH_2, CH_2CH_2 or $CH_2CH_2CH_2$ and L is a suitable leaving group, such as Cl, Br, I or tosyl, in the presence of a suitable base, such as LDA or NaH under standard conditions, to give a compound of the general formula XXXI,



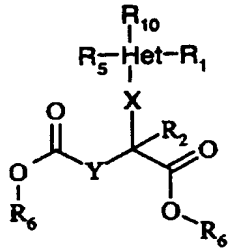
XXXI

b) hydrolysing a compound of the general formula XXXI, for example by treatment with aqueous NaOH or aqueous TFA under standard conditions to give a compound of the general formula II, wherein R_1, R_2, R_5, R_6, R_9, R_{10}, X, Y, Z and Het are as defined above, and R_3 is $COOR_6$ and R_4 is a  -group.

Process G

Process G for manufacture of compounds with the general formula II, wherein R_1, R_2, R_5, R_6, R_8, R_{10}, X, Z and Het are as defined above, Y is a single bond, R_3 is $COOR_6$, and R_4 is a  -group, comprises the following steps:

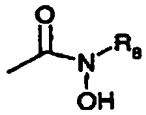
a) Compounds of the general formula XXXI



XXXI

can be reacted with compounds of the general formula XXXII

$$HR_8NOH \qquad \text{XXXII}$$

wherein R_8 is as defined in formula II, in the presence of suitable reagents, such as DCC/DMAP, under standard conditions, to give compounds of the general formula II, wherein R_1, R_2, R_5, R_6, R_8, X, Z and Het are as defined above, Y is a single bond, R_3 is $COOR_6$, and R_4 is a  -group.

Thrombin inhibitors

Thrombin inhibitors referred to in this application include but are not limited to low molecular weight thrombin inhibitors. The thrombin inhibitors are suitably low molecular weight peptide-based thrombin inhibitors. The term "low molecular weight peptide-based thrombin inhibitors" will be well understood by one skilled in the art to include thrombin inhibitors with one to four peptide linkages, and/or with a molecular weight below about 1,000, and includes those described generically as well as specifically in the review paper by Claesson in Blood Coagul. Fibrin. (1994) 5, 411, as well as those disclosed in US Patent No. 4,346,078; International Patent Applications WO 97/23499, WO 97/02284, WO97/46577, WO 98/01422, WO 93/05069, WO93/11152, WO 95/23609, WO 95/35309, WO 96/25426, WO 94/29336, WO 93/18060 and WO 95/01168; and European Patent Applications EP 623 596, EP 648 780, EP 468 231, EP 559 046, EP 641 779, EP 185 390, EP 526 877, EP 542 525, EP 195 212, EP 362 002, EP 364 344, EP 530 167, EP 293 881, EP 686 642, EP 669 317 and EP 601 459.

In the present application, thrombin inhibitors include chemical modifications, such as esters, prodrugs and metabolites, whether active or inactive, and pharmaceutically acceptable salts of any of these.

Preferred low molecular weight peptide-based thrombin inhibitors include those known collectively as the "gatrans". Particular gatrans which may be mentioned include HOOC-CH_2-(R)Cha-Pic-Nag-H (known as inogatran) and HOOC-CH_2-(R)Cgl-Aze-Pab-H (known as melagatran) (see International Patent Application WO 93/11152 and WO 94/29336, respectively).

International Patent Application WO 97/23499 discloses a number of compounds which have been found to be useful as prodrugs of thrombin inhibitors. Said prodrugs have the general formula

$$R^aOOC\text{-}CH_2\text{-}(R)Cgl\text{-}Aze\text{-}Pab\text{-}R^b$$

wherein R^a represents H, benzyl or C_{1-10} alkyl, R^b (which replaces one of the hydrogen atoms in the amidino unit of Pab-H) represents OH, $OC(O)R^c$ or $C(O)OR^d$, R^c represents C_{1-17} alkyl, phenyl or 2-naphtyl and R^d represents C_{1-12} alkyl, phenyl, C_{1-3} alkylphenyl, or 2-naphtyl. Preferred compounds include EtOOC-CH_2-(R)Cgl-Aze-Pab-OH. The active thrombin inhibitors themselves are disclosed in WO 94/29336.

Pharmaceutical formulations

The present invention relates to pharmaceutical compositions containing a CPU inhibitor and a thrombin inhibitor, or pharmaceutically acceptable salts thereof, as active ingredients.

Preferred combinations of a CPU inhibitor and a thrombin inhibitor are those where the CPU inhibitor is a compound of general formula I or II and the thrombin inhibitor is HOOC-CH_2-(R)Cgl-Aze-Pab-H or prodrugs thereof, particularly EtOOC-CH_2-(R)Cgl-Aze-Pab-OH.

In the present invention, the formulation and/or kits of parts may comprise two or more CPU inhibitors in combination with a thrombin inhibitor, two or more thrombin inhibitors in combination with a CPU inhibitor or any combination thereof. Two or more inhibitors include combinations of an active ingredient and one of its prodrugs.

For clinical use, the CPU inhibitor and the thrombin inhibitor are formulated into a pharmaceutical formulation for oral, intravenous, subcutaneous, tracheal, bronchial, intranasal, pulmonary, transdermal, buccal, rectal, parenteral or some other mode of administration. The pharmaceutical formulation contains the inhibitors in admixture with a pharmaceutically acceptable adjuvant, diluent and/or carrier.

The total amount of active ingredients suitably is in the range of from about 0.1 % (w/w) to about 95 % (w/w) of the formulation, suitably from 0.5 % to 50 % (w/w) and preferably from 1 % to 25 % (w/w).

The molar ratio between the CPU inhibitor and the thrombin inhibitor may be in the range of from about 1000:1 to about 1:1000. The molar ratio between the CPU inhibitor and the thrombin inhibitor lies suitably in the range of from 300:1 to 1:300, and preferably from 50:1 to 1:50.

In the preparation of the pharmaceutical formulations of the present invention the active ingredients may be mixed with solid, powdered ingredients, such as lactose, saccharose, sorbitol, mannitol, starch, amylopectin, cellulose derivatives, gelatin, or another suitable ingredient, as well as with disintegrating agents and lubricating agents such as magnesium stearate, calcium stearate, sodium stearyl fumarate and polyethylene glycol waxes. The mixture may then be processed into granules or pressed into tablets.

The active ingredients may be separately premixed with the other non-active ingredients, before being mixed to form a formulation. The active ingredients may also be mixed with each other, before being mixed with the non-active ingredients to form a formulation.

Soft gelatine capsules may be prepared with capsules containing a mixture of the active ingredients of the invention, vegetable oil, fat, or other suitable vehicle for soft gelatine capsules. Hard gelatine capsules may contain granules of the active ingredients. Hard gelatine capsules may also contain the active ingredients in combination with solid powdered ingredients such as lactose, saccharose, sorbitol, mannitol, potato starch, corn starch, amylopectin, cellulose derivatives or gelatine.

Dosage units for rectal administration may be prepared (i) in the form of suppositories which contain the active substance mixed with a neutral fat base; (ii) in the form of a gelatine rectal capsule which contains the active substance in a mixture with a vegetable oil, paraffin oil or other suitable vehicle for gelatine rectal capsules; (iii) in the form of a

ready-made micro enema; or (iv) in the form of a dry micro enema formulation to be reconstituted in a suitable solvent just prior to administration.

Liquid preparations may be prepared in the form of syrups or suspensions, e.g. solutions or suspensions containing the active ingredients and the remainder consisting, for example, of sugar or sugar alcohols and a mixture of ethanol, water, glycerol, propylene glycol and polyethylene glycol. If desired, such liquid preparations may contain coloring agents, flavoring agents, preservatives, saccharine and carboxymethyl cellulose or other thickening agents. Liquid preparations may also be prepared in the form of a dry powder to be reconstituted with a suitable solvent prior to use.

Solutions for parenteral administration may be prepared as a solution of a formulation of the invention in a pharmaceutically acceptable solvent. These solutions may also contain stabilizing ingredients, preservatives and/or buffering ingredients. Solutions for parenteral administration may also be prepared as a dry preparation to by reconstituted with a suitable solvent before use.

The dose of the compound to be administered will depend on the relevant indication, the age, weight and sex of the patient and may be determined by a physician. The dosage will preferably be in the range of from 0.01 mg/kg to 10 mg/kg.

The typical daily dose of the active ingredients varies within a wide range and will depend on various factors such as the relevant indication, the route of administration, the age, weight and sex of the patient and may be determined by a physician. In general, oral and parenteral dosages will be in the range of 0.1 to 1,000 mg per day of total active ingredients.

In the present invention, "a therapeutically effective total amount" relates to a total amount of the CPU inhibitor and the thrombin inhibitor which when given in combination gives a therapeutic effect, even though each amount when given separately may be less than the therapeutically effective amount.

Medical and pharmaceutical use

Also provided according to the present invention are formulations and kits of parts for use in medical therapy; the use of formulations of the present invention in the manufacture of medicaments for use in the treatment or prophylaxis of a condition in which inhibition of thrombin and/or inhibition of CPU are required or desired, and methods of medical treatment or prophylaxis comprising the administration of a therapeutically effective total amount of a CPU inhibitor and a thrombin inhibitor of the present invention to a patient suffering from, or susceptible to, a condition in which inhibition of thrombin and/or inhibition of CPU are required or desired.

The CPU inhibitor and the thrombin inhibitor can be administered sequentially, separately and/or simultaneously. Furthermore, the CPU inhibitor can be administered prior to the administration of the thrombin inhibitor or vice versa.

The term 'medical therapy' as used herein is intended to include prophylactic, diagnostic and therapeutic regimens carried out in vivo or ex vivo on humans or other mammals.

The formulations of the invention contain an inhibitor of CPU and an inhibitor of thrombin, either as such or, in the case of prodrugs, after administration. The formulations of the invention are thus expected to be useful in those conditions where inhibition of CPU and/or inhibition of thrombin are beneficial, such as in the treatment or prophylaxis of thrombosis and hypercoagulability in blood and tissues of mammals, including man.

It is known that hypercoagulability may lead to thromboembolic diseases. Conditions associated with hypercoagulability and thromboembolic diseases which may be mentioned include protein C resistance and inherited or aquired deficiences in antithrombin III, protein C, protein S and heparin cofactor II. Other conditions known to be associated with hypercoagulability and thromboembolic disease include circulatory and septic shock, circulating antiphospholipid antibodies, hyperhomocysteinemia, heparin induced

thrombocytopenia and defects in fibrinolysis. The formulations of the invention are thus indicated both in the therapeutic and/or prophylactic treatment of these conditions. The formulations of the invention are further indicated in the treatment of conditions where there is an undesirable excess of proCPU/CPU.

Particular disease states which may be mentioned include the therapeutic and /or prophylactic treatment of venous thrombosis and pulmonary embolism, arterial thrombosis (e.g. in myocardial infarction (MI), unstable angina, thrombosis-based stroke and peripheral arterial thrombosis) and systemic embolism usually from the atrium during arterial fibrillation or from the left ventricle after transmural myocardial infarction.

Moreover, the formulations of the invention are expected to have utility in prophylaxis of re-occlusion and restenosis (*i.e.* thrombosis) after thrombolysis, percutaneous trans-luminal angioplasty (PTA) and coronary bypass operations; the prevention of re-thrombosis after microsurgery and vascular surgery in general.

Further indications include the therapeutic and/or prophylactic treatment of disseminated intravascular coagulation caused by bacteria, multiple trauma, intoxication or any other mechanism, fibrinolytic treatment when blood is in contact with foreign surfaces in the body, such as vascular grafts, vascular stents, vascular catheters, mechanical and biological prosthetic valves or any other medical device, and fibrinolytic treatment when blood is in contact with medical devices outside the body, such as during cardiovascular surgery using a heart-lung machine or in haemodialysis.

EXAMPLES

The following Examples are intended to illustrate, but in no way limit the scope of the invention.

MATERIALS AND METHODS

Materials

Batroxobin No. 520 CRY (American Diagnostica Inc., Greenwich, CT, USA) was kept at 8°C both while lyophilized and when dissolved in saline to a concentration of 10 BU/ml. It was used within 5 days in the experiments. The remaining drug was freezed and kept at -20°C for a maximum of 30 days.

Endotoxin L-3129 (Sigma, St. Louis, MO, USA) was dissolved in saline to a concentration of 50 µg/ml. A fresh solution was prepared for each experiment.

^{125}I-labelled human fibrinogen (Amersham, Little Chalfont, Buck.,UK) was stored at 8°C while lyophilized. After dissolving in saline/aq.dest. (80/20 v/v) to a stock solution of approx. 0.8 MBq/ml (20 µCi/ml) it was divided into aliquots (one for each experiment), quickly freezed and stored at -20°C. It was used in the experiments within 14 days after the production date.

Inactin, thiobutabarbital sodium salt (Research Biochemicals International, Natick, USA) was dissolved in saline/aq.dest. (50/50 v/v) to a concentration of 50 mg/ml.

The thrombin inhibitor was dissolved in 40% (w/w) cyclodextrin (HPβCD) to a concentration of 5 mg/ml and stored at -20°C. It was further diluted with 40% cyclodextrin before use in the different experiments. In the studies the drug was administered subcutaneously.

The CPU inhibitor was dissolved in deoxygenated saline. The saline was deoxygenated by ultrasonication for 20 min and subsequently by passing nitrogen for another 20 min. The solutions were always fresh-made for each experiment. The CPU inhibitor was administered to the rats as an i.v. infusion for 5 min.

Animals

Male rats of the Sprague Dawley strain (Charles River, Sweden) with a body weight of 325 - 425 g were used in these experiments. The animals were allowed to accommodate at AstraZeneca R&D, Mölndal, Sweden for 1 week before use.

Animal preparation

Anaesthesia was induced and maintained by Inactin 100 mg/kg i.p.

Two catheters were inserted in the left jugular vein, one for administration of ^{125}I-fibrinogen and batroxobin and the other for administration of endotoxin and CPU inhibitor or their corresponding vehicles. A third catheter was inserted in the carotid artery for blood sampling and measurement of the mean arterial blood pressure and heart rate. To avoid blood clots in the arterial catheter a slow saline infusion (approx. 1.0 ml/kg*h) was maintained throughout the experiment. The rats were tracheotomized in order to facilitate spontaneous breathing. The body temperature was monitored and maintained at 38°C by external heating.

Experimental protocol

The animals were given an i.v. injection of endotoxin (50 µg/kg) at the start of the experiment. Two hours later the thrombin inhibitor or its vehicle was injected subcutaneously and another 15 min later ^{125}I-fibrinogen (0.04 MBq) was given as an i.v. injection. An infusion lasting for five minutes of the CPU-inhibitor or its vehicle was started after 5 min. After 5 min, batroxobin (10 BU/kg) was given as an i.v. bolus injection for one minute.

Blood samples for determination of ^{125}I-content were taken in all experiments just before and 5, 20 and 30 min after the batroxobin administration. At the end of the experiment the lungs were excised, the tissue was gently washed in saline and thereafter blotted and weighed. The ^{125}I-content in the tissue and blood samples was determined, using a Wallac 1282 Compugamma counter. The ^{125}I-concentration in plasma and in the lungs was

expressed as a percentage of the radioactivity in the reference arterial blood sample taken just prior to the batroxobin injection.

Example 1

The CPU inhibitor mergetpa (2-mercaptomethyl-3-guanidinoethylthiopropanoic acid) known e.g. from Biochimica & Biophysica Acta 1034 (1990) 86-92 was used in combination with the thrombin inibitor inogatran.

The results of the experiments are evident from Table I. The results are expressed as the remaining fibrin in the lung after treatment, i.e. 100 % is equal to no effect. n denotes the number of experiments (rats).

TABLE I

Effect on lung fibrin deposition in the anaesthetized rat in experiments with mergetpa and/or inogatran

CPU inhibitor		Thrombin inhibitor		CPU inhibitor + Thrombin inhibitor	
Dose (mg/kg)	Remaining fibrin after treatment (%±SD)	Dose (mg/kg)	Remaining fibrin after treatment (%±SD)	Dose (mg/kg)	Remaining fibrin after treatment (%±SD)
0.5	----------	0.25	----------	0.5 + 0.25	100±23.9 n = 6
1.0	----------	0.50	----------	1.0 + 0.50	62±21.9 n = 6
2.0	90±18.1 n = 6	1.0	94±20.2 n = 6	2.0 + 1.0	26±13.3 n = 9
5.0	37±19.3 n = 6	1.5	72±23.6 n = 6	----------	----------
8.0	23±17.3 n = 6	2.5	41±20.3 n = 6	----------	----------
----------	----------	7.5	39±15.9 n = 6	----------	----------

It is evident from Table I, that administration of the combination of a CPU inhibitor and a thrombin inhibitor is superior to administration of either inhibitor as regards remaining fibrin after treatment.

Example 2

The CPU inhibitor (3-(6-amino-pyridin-3-yl)-2-mercaptomethyl-propanoic acid) (Compound A) was used in combination with the thrombin inibitor melagatran.

The results of the experiments are evident from Table II. The results are expressed as the remaining fibrin in the lung after treatment, i.e. 100 % is equal to no effect. n denotes the number of experiments (rats).

TABLE II

Effect on lung fibrin deposition in the anaesthetized rat in experiments with compound A and/or melagatran

CPU inhibitor		Thrombin inhibitor		CPU inhibitor +	Thrombin inhibitor
Dose (mg/kg)	Remaining fibrin after treatment (%±SD)	Dose (mg/kg)	Remaining fibrin after treatment (%±SD)	Dose (mg/kg)	Remaining fibrin after treatment (%±SD)
0.05	100±25.8 n = 8	0.1	97±12.4 n = 6	0.05 + 0.1	75±35.2 n = 5
0.07	88±23.2 n = 4	0.15	77±22.4 n = 4	0.07 + 0.15	19±13.9 n = 8
0.1	87±50.6 n = 6	0.25	62±17.0 n = 2	----------	----------
0.5	49±30.0 n = 7	0.75	31±13.3 n = 4	----------	----------
1	15±9.3 n = 4	1.5	55±21.7 n = 4	----------	----------
5	7±1.4 n = 2	----------	----------	----------	----------

It is evident from Table II, that administration of the combination of a CPU inhibitor and a thrombin inhibitor is superior to administration of either inhibitor as regards remaining fibrin after treatment.

Abbreviations

Ac = acetate

AIBN = α,α´-azoisobutyronitrile

Aze = (S)-azetidine-2-carboxylic acid

Bn = benzyl

Bu = butyl

BU = batroxobin units as defined by American Diagnostica Inc., USA)

Bz = benzoyl

Cha = β-cyclohexyl alanine

Cgl = (S)-cyclohexyl glycine

DCC = dicyclohexylcarbodiimide

DIAD = diisopropyl azodicarboxylate

DIPEA = diisopropylethylamine

DMAP = N,N-dimethyl amino pyridine

EDC = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide

EtOH = ethanol

HMDS = hexamethyl disilazane

HOAc = acetic acid

HOBt = 1-hydroxybenzotriazol

HPβCD = hydroxypropyl β-cyclodextrin

i.p. = intraperitoneal

i.v. = intravenous

KHMDS = potassium bis(trimethylsilyl)amide

LDA = lithium diisopropylamide

Me = methyl

MeOH = methanol

Nag = noragmatine

Pab-H = 1-amidino-4-aminomethyl benzene

Ph = phenyl

Pic = pipecolinic acid

PMB = 4-methoxybenzyl

PyBOP = (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate

SD = standard deviation

TEA = triethylamine

TFA = trifluoroacetic acid

THF = tetrahydrofuran

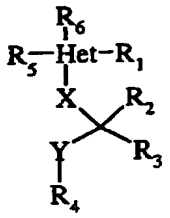
TMSCN = trimethylsilylcyanide

Tos(yl) = toluene-4-sulfonyl

CLAIMS

1. A pharmaceutical formulation, comprising:
(i) an inhibitor of carboxypeptidase U or a pharmaceutically acceptable salt thereof, and
(ii) a thrombin inhibitor or a pharmaceutically acceptable salt thereof, in admixture with a pharmaceutically acceptable adjuvant, diluent and/or carrier.

2. The pharmaceutical formulation according to claim 1, wherein the inhibitor of carboxypeptidase U is a compound of general formula I



(I)

or a pharmaceutically acceptable salt thereof, wherein

R_1 represents H, OH, halogen, C_1-C_6 alkyl, C_1-C_6 alkylaryl, C_1-C_6 alkoxy, cyano, NO_2, SH, $N(R_8)_2$, or CO-C_1-C_6 alkyl,

R_2 represents H, OH, halogen, or C_1-C_6 alkyl,

R_3 represents $COOR_7$, SO_2R_7, $S{=}O(OR_7)$, $P{=}O(OR_7)_2$, $B(OR_7)_2$, $P{=}OR_7(OR_7)$, tetrazole or any carboxylic acid isostere,

R_4 represents SH, S-CO- C_1-C_6 alkyl, S-CO-alkylaryl or S-CO-aryl,

R_5 represents H, OH, halogen, C_1-C_6 alkyl, C_1-C_6 alkylaryl, C_1-C_6 alkoxy, cyano, NO_2, SH, $N(R_8)_2$, or CO-C_1-C_6 alkyl,

R_6 represents H, OH, halogen, C_1-C_6 alkyl, C_1-C_6 alkylaryl, C_1-C_6 alkoxy, cyano, NO_2,

SH, $N(R_8)_2$, or $CO\text{-}C_1\text{-}C_6$ alkyl,

R_7 represents H, $C_1\text{-}C_6$ alkyl, $C_1\text{-}C_6$ alkylaryl, or aryl,

R_8 represents H or $C_1\text{-}C_6$ alkyl,

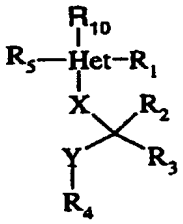
X represents O, S, $C(Z)_2$, N(Z), NR_8CO or $CONR_8$,

Y represents CH_2, or CH(Z),

Z represents H, $C_1\text{-}C_6$ alkyl, aryl or $C_1\text{-}C_6$ alkylaryl, and

Het represents a 4-, 5-, or 6-membered aromatic or alifatic heterocyclic group containing at least one nitrogen, oxygen or sulphur atom or a 4-, 5-, or 6-membered aromatic or alifatic carbocyclic group.

3. The pharmaceutical formulation according to claim 1, wherein the inhibitor of carboxypeptidase U is a compound of general formula II



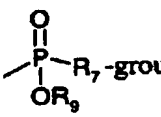
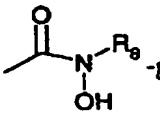
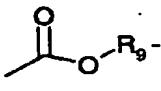
(II)

or a pharmaceutically acceptable salt thereof, wherein

R_1 represents H, OH, halogen, $C_1\text{-}C_6$ alkyl, $C_1\text{-}C_6$ alkylaryl, $C_1\text{-}C_6$ alkoxy, cyano, NO_2, SH, $N(R_8)_2$, $CO\text{-}C_1\text{-}C_6$ alkyl, or a guanidino group,

R_2 represents H, OH, halogen, or $C_1\text{-}C_6$ alkyl,

R_3 represents $COOR_6$, SO_2R_6, SO_3R_6, $P{=}O(OR_6)_2$, $B(OR_6)_2$, $P{=}OR_6(OR_6)$, tetrazole or any carboxylic acid isostere,

R_4 represents a  -group, or a  -group, or a  -group,

R_5 represents H, OH, halogen, C_1-C_6 alkyl, C_1-C_6 alkylaryl, C_1-C_6 alkoxy, cyano, NO_2, SH, $N(R_8)_2$, CO-C_1-C_6 alkyl, or a guanidino group,

R_6 represents H, C_1-C_6 alkyl, C_1-C_6 alkylaryl, or aryl,

R_7 represents C_1-C_6 alkyl, C_1-C_6 alkylaryl, or aryl, or a dipeptide or an aminoacid residue, both optionally N-protected,

R_8 represents H, C_1-C_6 alkyl, or C_1-C_6 alkylaryl,

R_9 represents H, C_1-C_6 alkyl, C_1-C_6 alkylaryl, aryl, or $OC(Z)_2OCOR_8$,

R_{10} represents H, OH, halogen, C_1-C_6 alkyl, C_1-C_6 alkylaryl, C_1-C_6 alkoxy, cyano, NO_2, SH, $N(R_8)_2$, CO-C_1-C_6 alkyl, or a guanidino group,

X represents O, S, CH_2, CH_2CH_2, $CH_2CH_2CH_2$, NH, CH(Z) or N(Z),

Y represents O, CH_2, or CH(Z), or a single bond,

Z represents H, C_1-C_6 alkyl, C_1-C_6 alkylaryl, or aryl, and

Het represents a 4-, 5-, or 6-membered aromatic or alifatic heterocyclic group containing at least one nitrogen, oxygen or sulphur atom, a 4-, 5-, or 6-membered aromatic or alifatic carbocyclic group or a single bond.

4. The pharmaceutical formulation according to any previous claim, wherein the thrombin inhibitor is a low molecular weight thrombin inhibitor.

5. The pharmaceutical formulation according to claim 4, wherein the low molecular weight thrombin inhibitor is an oligopeptide with one to four peptide linkages.

6. The pharmaceutical formulation according to claim 5, wherein the low molecular weight thrombin inhibitor is HOOC-CH_2-(R)Cgl-Aze-Pab-H or prodrugs thereof.

7. The pharmaceutical formulation according to claim 6, wherein the prodrug is EtOOC-CH_2-(R)Cgl-Aze-Pab-OH.

8. The pharmaceutical formulation according to any previous claim, wherein the molar ratio between the inhibitor of carboxypeptidase U and the thrombin inhibitor lies in the range of from about 1000:1 to about 1:1000, preferably from 50:1 to 1:50.

9. A kit of parts comprising:
(i) a vessel containing an inhibitor of carboxypeptidase U, or a pharmaceutically acceptable salt thereof;
(ii) a vessel containing a thrombin inhibitor, or a pharmaceutically acceptable salt thereof; and instructions for the sequential, separate and/or simultaneous administration of the inhibitors (i) and (ii) to a patient in need thereof.

10. The kit of parts according to claim 9, wherein the inhibitor of carboxypeptidase U is a compound as defined in claim 2 or 3.

11. The kit of parts according to claims 9 or 10, wherein the thrombin inhibitor is a low molecular weight thrombin inhibitor.

12. The kit of parts according to claim 11, wherein the low molecular weight thrombin inhibitor is an oligopeptide with one to four peptide linkages.

13. The kit of parts according to claim 12, wherein the low molecular weight thrombin inhibitor is HOOC-CH_2-(R)Cgl-Aze-Pab-H or prodrugs thereof.

14. The kit of parts according to claim 13, wherein the prodrug is EtOOC-CH_2-(R)Cgl-Aze-Pab-OH.

15. The kit of parts according to any one of claims 9 to 14, wherein the molar ratio between the inhibitor of carboxypeptidase U and the thrombin inhibitor lies in the range of from about 1000:1 to about 1:1000, preferably from 50:1 to 1:50.

16. A kit of parts comprising:
(i) a pharmaceutical formulation containing an inhibitor of carboxypeptidase U, or a pharmaceutically acceptable salt thereof, in admixture with a pharmaceutically acceptable adjuvant, diluent and/or carrier; and
(ii) a pharmaceutical formulation containing a thrombin inhibitor, or a pharmaceutically acceptable salt thereof, in admixture with a pharmaceutically acceptable adjuvant, diluent and/or carrier;
which inhibitors (i) and (ii) are each provided in a form that is suitable for administration in conjunction with the other.

17. The kit of parts according to claim 16, wherein inhibitors (i) and (ii) are suitable for sequential, separate and/or simultaneous administration.

18. The kit of parts according to claim 16 or 17, wherein the inhibitor of carboxypeptidase U is a compound as defined in claim 2 or 3.

19. The kit of parts according to any one of claims 16 to 18, wherein the thrombin inhibitor is a low molecular weight thrombin inhibitor.

20. The kit of parts according to claim 19, wherein the low molecular weight thrombin inhibitor is an oligopeptide with one to four peptide linkages.

21. The kit of parts according to claim 20, wherein the low molecular weight thrombin inhibitor is HOOC-CH_2-(R)Cgl-Aze-Pab-H or prodrugs thereof.

22. The kit of parts according to claim 21, wherein the prodrug is EtOOC-CH_2-(R)Cgl-Aze-Pab-OH.

23. The kit of parts according to any one of claims 16 to 22, wherein the molar ratio between the inhibitor of carboxypeptidase U and the thrombin inhibitor lies in the range of from about 1000:1 to about 1:1000, preferably from 50:1 to 1:50.

24. A formulation according to any one of claims 1 to 8, or a kit of parts according to any one of claims 9 to 23, for use in medical therapy.

25. A method for treatment or prophylaxis of a patient suffering from, or susceptible to, a condition in which inhibition of thrombin and/or inhibition of carboxypeptidase U are required or desired, which method comprises administering to the patient a therapeutically effective total amount of

(i) an inhibitor of carboxypeptidase U, or a pharmaceutically acceptable salt thereof, in admixture with a pharmaceutically acceptable adjuvant, diluent and/or carrier; in conjunction with

(ii) a thrombin inhibitor, or a pharmaceutically acceptable salt thereof, in admixture with a pharmaceutically acceptable adjuvant, diluent and/or carrier.

26. The method according to claim 25, wherein the administration of inhibitors (i) and (ii) is sequential, separate and/or simultaneous.

27. The method according to claim 26, wherein inhibitor (i) is administered prior to the administration of component (ii) or vice versa.

28. The method according to any one of claims 25 to 27, wherein the inhibitor of carboxypeptidase U is a compound as defined in claim 2 or 3.

29. The method according to any one of claims 25 to 28, wherein the thrombin inhibitor is a low molecular weight thrombin inhibitor.

30. The method according to claim 29, wherein the low molecular weight thrombin inhibitor is an oligopeptide with one to four peptide linkages.

31. The method according to claim 30, wherein the low molecular weight thrombin inhibitor is HOOC-CH_2-(R)Cgl-Aze-Pab-H or prodrugs thereof.

32. The method according to claim 31, wherein the prodrug is EtOOC-CH_2-(R)Cgl-Aze-Pab-OH.

33. The method according to any one of claims 25 to 32, wherein the molar ratio between the inhibitor of carboxypeptidase U and the thrombin inhibitor lies in the range of from about 1000:1 to about 1:1000, preferably from 50:1 to 1:50.

34. A method for treatment or prophylaxis of a patient suffering from, or susceptible to, a condition in which inhibition of thrombin and/or inhibition of carboxypeptidase U are required or desired, which method comprises administering to the patient a formulation as defined in any one of claims 1 to 8.

35. The use of a formulation according to any one of claims 1 to 8, in the manufacture of a medicament for the treatment or prophylaxis of a condition in which inhibition of thrombin and/or inhibition of carboxypeptidase U are required or desired.

36. The use according to claim 35, wherein the inhibitor of carboxypeptidase U is a compound as defined in claim 2 or 3.

37. The use according to claim 35 or 36, wherein the thrombin inhibitor is a low molecular weight thrombin inhibitor.

38. The use according to claim 37, wherein the low molecular weight thrombin inhibitor is an oligopeptide with one to four peptide linkages.

39. The use according to claim 38, wherein the low molecular weight thrombin inhibitor is HOOC-CH_2-(R)Cgl-Aze-Pab-H or prodrugs thereof.

40. The use according to claim 39, wherein the prodrug is EtOOC-CH_2-(R)Cgl-Aze-Pab-OH.

41. The use according to any one of claims 35 to 40, wherein the molar ratio between the inhibitor of carboxypeptidase U and the thrombin inhibitor lies in the range of from about 1000:1 to about 1:1000, preferably from 50:1 to 1:50.

ABSTRACT

The present invention relates to pharmaceutical formulations of an inhibitor of carboxypeptidase U (CPU) and a thrombin inhibitor in admixture with a pharmaceutically acceptable adjuvant, diluent and/or carrier, as well as a kit of parts, a method for treatment and use of the formulations for the treatment or prophylaxis of a condition in which inhibition of CPU and/or inhibition of thrombin are required or desired.